Amphipod *Eohaustorius estuarius* Sediment Toxicity Test

1.0 OBJECTIVE

Many anthropogenic contaminants in aquatic systems become bound to particles and may subsequently accumulate in sediments. Sediment toxicity to marine or estuarine infaunal amphipods can be assessed using this Standard Operating Procedure (SOP). Observed effects may be related to the presence of contaminants or to naturally occurring factors. To correctly interpret toxicity results, a number of parameters should be analyzed, including sediment chemistry, grain size, and TOC, as well as the following water quality parameters: dissolved oxygen, pH, salinity, ammonia, sulfide, and temperature.

In this procedure, sediment collected from field stations is divided into replicate beakers in the laboratory and covered with dilution water, which is gently aerated. Twenty randomly selected amphipods are placed into each replicate container and allowed to burrow into the test sediments. Each beaker is monitored daily for aeration rate and amphipod emergence from the sediment (optional). After a 10-day exposure, the sediment is sieved to recover the amphipods, and live animals are counted to determine the percentage that survived the exposure. Sediment toxicity is characterized by the mean percent survival (± standard deviation) for each sediment sample. This can be compared to the survival observed in sediment from the amphipod collection site (home sediment), or in sediment from reference sites presumed to have similar natural characteristics but low contaminant concentrations.

2.0 EQUIPMENT

The following equipment is necessary to conduct the toxicity test at the Marine Pollution Studies Laboratory at Granite Canyon (MPSL). The word "clean" here and throughout this procedure means that the item has been cleaned according to the MPSL glassware cleaning procedures outlined in a separate standard operating procedure (MPSL SOP 1.3).

2.1 Test Initiation

- 1000-mL clean glass beakers (7 per sediment sample - 5 for test, 2 for water quality)
- Acrylic covers with airlines and tubing sleeves around clean Pasteur pipettes
- Clean polypropylene spoons to scoop sediment into containers (one per sediment sample)
- 1000-mL clean plastic tri-pour beakers (18) with covers for reference toxicant test
- 1000-mL volumetric flasks (2) and pipettes for reference toxicant dilutions
- Large clean culture tray to hold and acclimate *Eohaustorius* in home sediment
- Clean 15 cm tall buckets to hold screens during rough sorting
- Clean 500-µm screens to sort organisms
- Clean 250-mL crystallizing dishes to sort amphipods
- Clean glass tubes with squeeze bulbs and screens for sorting amphipods
- Hand counters
- Constant temperature room at 15°C

• Gloves and appropriate safety gear (see MPSL lab safety manual)

2.2 Test Termination

• Toxic 500-µm screens to sieve pods at test termination
• Toxic glass tubes with squeeze bulbs and screens for collecting amphipods
• Data Sheets and clipboards
• Pens (and towel to dry hands for writing)
• Gloves and appropriate safety gear (see MPSL lab safety manual)

2.3 Water Quality

• Meters and probes for measuring pH, dissolved oxygen
• Spectrophotometers for measuring sulfide and ammonia
• Refractometer for measuring salinity
• Thermometers (glass mercury thermometer and continuously recording chart thermometer)
• Centrifuge for isolation of porewater
• Graduated pipettes (10 ml) and hand pipette pump for water quality sampling
• Gloves and appropriate safety gear (see MPSL lab safety manual)

2.4 Dilution Water

In every step of this procedure, use water made from 1-µm filtered Granite Canyon seawater mixed with distilled water from the lab distiller. Store bought spring water may be substituted for distilled water when necessary. Leach seawater filters for a minimum of 24 hours before using.

Throughout this protocol use water with a salinity of 20‰ and a temperature of 15°C. The formula for calculating volumes of distilled and seawater is: $V_1S_1 = V_2S_2$. For example: V_1* (34‰) = (10 L) * (20‰). V_1 = 10 * 20/34 = 6 L of seawater mixed with 4 L of distilled water. Use this formula only as a guide, since Granite Canyon seawater salinity may vary. Measure the actual salinity, adding and mixing seawater or distilled water, as needed until salinity reaches 20 ± 1‰.

3.0 EXPERIMENTAL DESIGN

Sediment toxicity tests can be used as screening tools or as part of more comprehensive studies to assess sediment quality. Careful consideration must be given to site characteristics, reference site selection, field replication, choice of synoptic measures, seasonal factors, comprehensive planning and peer review to determine that study designs are adequate to meet program objectives.

This laboratory toxicity test consists of five replicate test beakers for each sediment sample. Beakers are arranged randomly, and each receives 20 randomly selected amphipods. The quality of test amphipods and testing conditions is determined through concurrent testing of reference toxicants (positive controls) and home (collection site) sediment (negative controls). Both are discussed in Section 7. Testing of reference sites is recommended to demonstrate the suitability of test sediments in the absence of toxic contaminant concentrations. Test conditions of temperature and photoperiod are controlled as indicated below, and pH, H_2S, NH_3, salinity, dissolved oxygen, and temperature are measured at the beginning and end of the exposure.

4.0 PREPARATION OF SEDIMENTS FOR TESTING

Label 1-L glass test beakers sequentially as indicated on the randomization sheet generated for the test. Arrange the labeled beakers using the randomization sheet. Label two additional sets of beakers with the sample number for water quality measurements at the initiation and termination of the test.

Remove test sediments from refrigerated storage and place samples on the lab bench prepared for distributing sediment into test beakers. Carry a small number of samples at a time to avoid injury and possible loss of samples to breakage. Minimize sample exposure to sunlight (never leave samples in direct sunlight), and schedule loading times to avoid prolonged sample exposure to temperatures above 15°C. Do not sieve, freeze, or allow test sediments to dry prior to testing. Remove large objects such as sticks or clams with forceps, and note their presence on the data sheet.

Using a separate clean polypropylene spoon for each sample, re-homogenize (stir) the sediment in the sample jar to thoroughly mix overlying water back into the sediment. Spoon 175 mL of sediment into each of the five test beakers, and the extra water quality beakers, forming a layer 2-cm deep. When the jar is empty, leave the spoon in the sample jar so it won't be used for other samples. Add water (20‰, 15°C) to the 750- mL level carefully so as not to disturb the sediment. Arrange the test beakers in numerical order on racks in the constant temperature room. Cover beakers with acrylic air racks and insert pipettes into the beakers to about 2 cm above the sediment. Adjust airflow to slightly more than one bubble per second, making sure aeration does not disturb the sediment surface. Allow the sediment to equilibrate at test temperature overnight. Handle reference site and test site sediments in the same manner.

5.0 CONTROLS

5.1 Home (Collection Site) Sediment Controls (Negative Controls)

Order home sediment from the amphipod supplier with the amphipods. Store home sediment in the refrigerator at 4 ± 3°C in the dark. Using a clean polypropylene spoon, distribute the home sediment into five test beakers. Sediment should form a layer 2-cm deep. Carefully add water (20‰, 15°C) to the 750-mL level.

5.2 Reference Toxicant Tests (Positive Controls)

Conduct a concurrent reference toxicant test each time a new batch of amphipods is used for testing sediments (at a minimum), or each time sediments are tested (standard procedure). The reference toxicant test is a 96-hour exposure using a water-only dilution series of cadmium chloride, and provides data on the relative sensitivity of each amphipod batch.

Prepare a stock solution of 100 mg Cd per liter by weighing 0.1630 grams of cadmium chloride ($CdCl_2$), and pouring the weighed solid into one liter of distilled water in a plastic volumetric flask. Cap tightly and mix thoroughly. Use the cadmium dilution sheet to prepare 6 concentrations of reference toxicant. Prepare each concentration 2 times and divide mixture into 3 replicate one-liter plastic beakers. Place the reference toxicant test containers in the constant temperature room, cover, and allow to equilibrate. Aeration is not necessary.

6.0 TEST INITIATION

6.1 Amphipod Acclimation

Order amphipods to arrive at least 2 days, but no longer than one week, before test initiation. Place sediment containing the amphipods in a clean (culture) tray containing water at a temperature and salinity that varies by no more than 3°C or 3‰ from transport conditions. Acclimate the amphipods to test temperature and salinity by changing temperature by no more than 3°C per day and salinity by no more than 10‰ per day. Adjust the salinity by mixing a new batch of water at the appropriate salinity and temperature, siphoning out the old overlying water, and pouring in the new water. Hold amphipods at test temperature and salinity for 48 hours prior to initiating sediment testing. Remove any dead or moribund animals visible at the sediment surface. Make sure water in the tray is constantly aerated. Check the amphipods daily and re-submerge any amphipods stuck at the air surface by gently pushing them with a clean glass rod or pipette. Monitor the health of amphipods by observing emergence and appearance. If more than 5% of the amphipods emerge and appear unhealthy during the 48 hours prior to the test, reschedule the test and immediately arrange for another amphipod shipment.

6.2 Randomized Isolation of Amphipods

Using a 500-µm screen submerged in a half bucket filled with water (20‰, 15°C), sieve a portion of the sediment to isolate the amphipods. Using a pipette or screen, transfer the amphipods from the screen tube into a crystallizing dish filled with about 200 mL of water (20‰, 15°C). Only transfer animals that are healthy and moving. Count the amphipods carefully and make sure each dish has exactly 20 amphipods; this count is crucial for correct interpretation of mortality at the end of the test. Maintain the dishes at 15°C during isolation by keeping them in the constant temperature room. Load the amphipods into the test containers without delay to minimize time spent out of the sediment.

6.3 Loading Amphipods into Test Containers

Make sure the amphipods in the crystallizing dishes are all healthy and are at the same temperature as the test containers. Take a crystallizing dish that you know contains exactly 20 amphipods, and swirl it gently to free any animals stuck or clinging to the dish. Quickly and carefully pour the entire contents of the dish into a test beaker, taking care not to disturb the sediment. Be sure all amphipods have gone into the test beaker. Wash any remaining amphipods from the dish into the beaker with a squirt bottle (20‰, 15°C). Repeat this process until all beakers (or the desired subset) are loaded. Place the acrylic covers over each set of beakers on a shelf, and check pipette placement and airflow. After approximately one hour, check the beakers for amphipods that are in poor condition and have not buried themselves. Replace injured or stressed amphipods.

7.0 MONITORING THE TOXICITY TEST

7.1 Counting Amphipod Emergence

Test duration is 10 days. Check all test containers daily, adjust airflow if necessary, and record the number of amphipods that are visible on the sediment surface, in the water column, or at the air surface (optional). Push any amphipods trapped at the air surface back under water with a pipette.

7.2 Measuring Water Quality in Test Containers

Measure salinity, temperature, dissolved oxygen, pH, and ammonia in the overlying water of the water quality replicate from each sample at the beginning and end of the test. Measure pH, salinity, ammonia, and hydrogen sulfide in porewater from the same replicate of each sample at the beginning and end of the test. Sample the test solutions as described below, and measure each parameter following the MPSL standard operating procedure for each measurement.

On the first day of the test, before the amphipods are loaded into the beakers, use a clean graduated 10-mL pipette to remove a water sample from within 1 cm of the sediment surface for water quality measurement. Take water quality samples from extra replicates set up for that purpose. Sample as close to the sediment as possible without disturbing the sediment or drawing fine particles into the pipette. Deliver the sample into water quality containers that are pre-labeled with the sample number. Before sampling the next beaker, rinse the pipette by drawing two successive volumes of distilled water and discarding. After overlying water has been sampled, the water is then poured off and a small spatula is used to sample the sediment from the beaker into a 50-mL centrifuge tube. Spin the tube in the centrifuge for 30 minutes at 2500 G (4º C.). Remove pore water from the tube with a 10-mL pipette using the above procedure. Porewater should be sampled for ammonia, pH salinity, and sulfide. Store the sulfide sample in a 4.5 mL labeled vial with preservative.

Repeat this sampling procedure on the last day of the test, taking water quality samples from 1 cm above the sediment in the sixth replicate beaker. If the study design does not require additional water quality make sure to get

samples from all treatments before they are sieved to remove amphipods. Before sampling, turn off the airflow and carefully remove the covers, airlines and pipettes from the beakers. Be sure not to draw any amphipods into the pipette while sampling. Identify which beakers these are and do not sieve them, set them aside for porewater extraction and measurement of interstitial ammonia, sulfide, pH, and dissolved oxygen.

8.0 TERMINATING THE TOXICITY TEST

After 10 days of exposure, amphipods are removed from the sediment to determine rates of survival. After removing covers and airlines, sampling for water quality, setting aside beakers for porewater extraction and counting emergence, carry beakers to the sieving table.

8.1 Preparation for Sieving

Before sieving, make sure the data sheet is ready on a clipboard, with a towel and pen next to it, in a place where it will be very difficult to go to the next beaker without remembering to write down your last count. Put on a lab coat, apron, boots, and gloves. Clear the water table of any old mud, if necessary, check water flows, and carefully examine your 500-µm screen to make sure there are no holes (>500µm).

8.2 Sieving, Recovering Amphipods, and Recording Data

Pour the entire contents of a test beaker onto the sieve, hosing down the beaker walls to remove all particles larger than an amphipod and to make it easier to clean the beakers later. Spray seawater over the mud in the screen to wash particles away from the remaining amphipods. Break up any clods or mats, and continue spraying until all the fine sediment particles are removed. Quickly submerge the screen, catching the amphipods in the water surface tension. Use a screen to transfer all visible amphipods to a crystallizing dish, and then repeat the washing and submerging process to recover more amphipods. Continue this procedure until you are certain that you have recovered every amphipod. Count the number of live amphipods you have collected in the dish, and record that number immediately, before doing anything else. Missing animals and animals that do not respond to a probe are considered dead. Put empty beakers in a tray, and haul them to the dish room, where they will be washed according to the glassware cleaning SOP.

Take the completed data sheet to the office for data entry and analysis. Notify the data analyst that the data has arrived. Make sure the data sheets are placed in the proper location and that the person keeping track of the data knows where it is.

9.0 TEST ACCEPTABILITY AND PERFORMANCE CRITERIA

This toxicity test procedure is considered unacceptable if amphipod survival in home sediment controls is less than 90%, or if the survival in any single replicate is less than 80%. Tests with temperature, salinity, or dissolved oxygen measurements outside the specified ranges, may be considered conditionally acceptable based on the project

officer's best professional judgment. Acceptable temperatures range from 14° to 16° C; acceptable salinities range from 17‰ to 23‰; acceptable dissolved oxygen concentrations range from 5.35 to 8.92 mg/L.

10.0 REFERENCES

U.S. Environmental Protection Agency. 1994. Methods for assessing the toxicity of sediment-associated contaminants with estuarine and marine amphipods. EPA/600/R-94/025. Office of Research and Development, Washington D.C.

11.0 TEST SUMMARY

Test Duration	10 days
Endpoint	Survival
Renewals	None
Organism Source	Wild caught adults of consistent size
Acclimation	10‰ and 3°C per day to 20‰ and 15°C
Salinity	20 ± 3‰ recommended
Dissolved oxygen	>5.35 mg/L recommended
Temperature:	15 ± 1°C daily mean required
	15 ± 3°C instantaneous required
Dilution/Overlying water	1-µm filtered seawater with distilled water mixed to 20‰
Aeration	Through a pipette 2 cm above sediment, >1 bubble/second.
Lighting	Constant illumination.
Replication	5 replicates per sample, plus 2 for water quality.
Test Containers	1000-mL Beakers
Loading	20 amphipods per beaker
Overlying Water Quality	pH, dissolved oxygen, temperature, salinity, NH_3
Interstitial Water Quality	pH, salinity, NH_3, H_2S
Reference Toxicant	Cadmium Chloride ($CdCl_2$)
Daily Monitoring	Check aeration, count emergence (optional)
Acceptability Criteria	Controls: Mean >90%, >80% in replicates